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(54) Title: USES OF TGF- β RECEPTOR FRAGMENT AS A THERAPEUTIC AGENT (57) Abstract A method of treating TGF- β excess is disclosed. The treatment is parenteral, oral or topical administration of TGF- β re- ceptor fragment. Particularly effective is a soluble receptor fragment which resembles the extracellular portion of TGF- β binding protein II.		

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USES OF TGF- β RECEPTOR FRAGMENT
AS A THERAPEUTIC AGENT

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DESCRIPTION

Technical Field

This invention relates to the fields of drug therapy and protein synthesis. A soluble TGF- β binding protein fragment is used to treat conditions characterized by an excess of TGF- β , including fibroproliferation and immunosuppression. The present invention also relates to recombinant expression of the binding protein fragment in prokaryotic and eukaryotic cells.

20

Technical Background

Transforming growth factor- β (TGF- β) represents a family of polypeptides, of which three are present in mammals, TGF- β 1, TGF- β 2 and TGF- β 3. These factors have global effects on cell growth and differentiation (Roberts and Sporn (1990) Handbk. Exp. Pharm. 95:419-58). There is a growing body of evidence that TGF- β also modulates the immune process (Wahl et al. (1989) Immunol. Today 10:258-61). In addition to stimulating the congregation of immune cells at the site of injury, TGF- β also provides strong positive feedback for its own continued synthesis (Kim et al. (1990) Mol. Cell. Biol. 10:1492-1497). These factors have led to the investigation of the role of TGF- β in immune and fibroproliferative disorders.

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In fibroproliferative diseases, TGF- β is becoming considered as a prominent factor. TGF- β is known 1) to stimulate cells to produce more proteins, including collagen, biglycan, decorin and fibronectin; 5 and 2) to inhibit enzymes which degrade these proteins. Thus, TGF- β can cause fibrous tissue to accumulate. For example, in diabetic nephropathy and human mesangial proliferative glomerulonephritis, both fibroproliferative diseases, a prominent and important pathological feature 10 is the accumulation of mesangial matrix (Mauer et al. (1984) J. Clin. Invest. 74: 1143-55). Likewise, postradiation fibrosis is characterized by excessive TGF- β , proliferation of fibroblasts and overproduction of connective tissue (Canney and Dean (199) Brit. J. Radiol. 15 63:620-23).

There have been several attempts to suppress the effects of TGF- β excess by administering antibody which is specific for TGF- β . In a pending patent application Serial No. 759,109, filed September 6, 1991, 20 also assigned to Celtrix Pharmaceuticals, Inc., monoclonal antibodies to TGF- β were shown to have affinity constants ranging from 1.6×10^7 L/mol to 3.4×10^8 L/mol in a competitive radioimmunoassay test. These monoclonal antibodies were suggested for use in treating 25 tumor cells that produce TGF- β to counteract the immunosuppressive effects of TGF- β . Another proposed use was treating metastatic cancers.

Border et al. (1990) Nature 346:371-74, found that antiserum against TGF- β suppressed experimentally 30 induced glomerulonephritis, which was characterized by mesangial proliferation. Border et al. reported that the antibodies to TGF- β which were raised in rabbits had 50% binding to TGF- β at a ratio of 1:6000 in a radioimmunoassay. Antibodies typically have a molecular 35 weight of at least 150 kilodaltons (kd).

More recently, Border et al. ((1992) Kidney Int. 41:566-570) mentioned that "[o]ther investigators have used our strategy of antagonizing TGF- β by administering anti-TGF- β in vivo. This approach has confirmed a causal role for TGF- β in pathological matrix accumulation by reducing scar formation in fetal skin, in the central nervous system following wounding and reduction of interstitial fibrosis in a model of acute lung injury." (p. 567) In a paper accepted for publication in J. Exp. Med., Wahl also cites TGF- β excess in "a spectrum of connective tissue disorders including rheumatoid arthritis, scleroderma, myelofibrosis, and hepatic, intraocular, and pulmonary fibrosis."

Anti-TGF- β antibodies have been administered to animals given an intraarticular injection of bacterial cell walls in an amount sufficient to cause the development of arthritis. One intraarticular injection of anti-TGF- β was sufficient to prevent arthritis. (Wahl (1992) J. Clin. Immunol. 12:1-14). In the J. Exp. Med. paper, Wahl et al. reported that antibody injected into a joint before systemic administration of streptococcal cell wall (SCW) resulted in a 75% decrease in joint inflammation. Even if the antibody were injected about two weeks after the SCW injection, at which time the inflammation had become chronic, there was still a significant benefit. Likewise, Goddard et al. ((1990) Cytokine 2: 149-55) found that TGF- β inhibited the growth of cultured synovial cells, which was reversed by administration of neutralizing antibodies.

TGF- β -specific antibodies also were injected into the margins of healing dermal wounds in adult rats. Control wounds (those with irrelevant antibody or TGF- β) all had scarring, but the antibody-treated wounds healed completely with normal strength but no scar formation. Shah et al., (1992) The Lancet 339: 213-14.

TGF- β -specific antibody has also been found to partially restore, at least in laboratory tests, the defective T-cell responses due to excess TGF- β production such as in patients with acquired immune deficiency syndrome (AIDS). (Kekow et al. (1990) Proc. Natl. Acad. Sci. 87:8321).

In rat prostate cancer, TGF- β 1 is overexpressed, compared to normal prostate tissue. It appears that TGF- β 1 enhances tumor growth by stimulating tumor cells (Steiner and Barrack (1992) Mol. Endocrinol. 6:15-25). Steiner and Barrack tested the effect of anti-TGF- β antibody on over-producing prostate cancer cells which had stopped growing. The antibody caused the prostate cancer cells to begin proliferating again. In mouse prostate cancer, both TGF- β 1 and 3 were elevated and were correlated with progression to malignancy and may even promote carcinoma (Merz et al. (1991) Mol. Endocrinol. 5:503-13).

Another way of suppressing TGF- β in experimental glomerulonephritis in rats, which is associated with TGF- β 1 excess, was a low-protein diet. Both the excreted nitrogen and the expressed TGF- β 1 decreased. (Okuda et al. (1991) Proc. Natl. Acad. Sci. U.S.A., 88:9765-69.)

In addition, TGF- β has natural inhibitors, including decorin and endoglin. Yamaguchi et al. ((1990) Nature 346:281-84) have proposed that decorin binds TGF- β and provides negative regulation of the TGF- β by interfering with TGF- β binding to a receptor. Decorin is highly glycosylated and has a molecular weight of about 46 kd and an affinity for TGF- β of about 10^{-8} to 10^{-9} M.

Endoglin also appears to bind TGF- β with an affinity constant of about 5×10^{-11} M. Endoglin also is highly glycosylated and has a molecular weight of about 90 kd.

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Previously, anti-TGF- β , decorin and endoglin have been suggested as inhibitors of TGF- β . However, these three proteins share the undesirable feature of high molecular weight (20-180 kd). Affinity constants for the antibody and decorin are relatively low, ranging from about 10^{-8} to 10^{-9} M. Moreover, administering antibodies from other species can cause cross-species reactions.

An improved inhibitor would have a much lower molecular weight and higher affinity. This combination of features would permit much lower doses and increase ease of administration. Moreover, a native protein would not cause cross-species reactions.

Nine classes of TGF- β receptors have been found. The main receptors on cells are Type I, Type II and Type III. A soluble form of the Type III receptor has been detected, and it binds TGF- β similarly to the membrane-bound Type III receptor (Andres et al. (1989) J. Cell. Biol. 109:3137-45).

The human Type II receptor has been cloned and codes for a protein of 563 amino acids (Lin et al. (1992) Cell, 68: 775-85). This protein contains three discreet domains: a 136-amino acid extracellular domain, a 30-amino acid hydrophobic transmembrane domain, and a 376-amino acid intracellular domain. The extracellular region binds TGF- β . The Type II receptor has a very high affinity for TGF- β , on the order of about 10^{-11} to 10^{-12} M. Furthermore, the Type II receptor is a native human protein, which should help avoid cross-species reactions. And finally, the region which binds TGF- β is only about 100 amino acids long. Therefore, its molecular weight is only a fraction of previously suggested inhibitors.

Production of recombinant heterologous proteins in prokaryotic host cells is essential to produce

commercially feasible amounts of protein. Unfortunately, bacterial host cells such as *E. coli* often are not ideal or simply cannot be used to produce such proteins. This is because the proteins, when over-expressed, form refractile, insoluble "inclusion bodies" and/or prove lethal to the cells. Inclusion bodies are found in the cytoplasm of the cell. Although inclusion bodies can be isolated from the cell by cell lysis and centrifugation, subsequent purification of the proteins involves dissolving the inclusion bodies and renaturing the proteins. Renaturation is not always effective or efficient. A variety of mechanisms have been sought to overcome these problems. However, none of the methods are ideal.

Purification of proteins produced in bacterial host cells has also proven to be problematic. In many cases, the proteins of interest, particularly when incorporated into inclusion bodies, co-purify with bacterial cell wall components which can be toxic.

Mammalian cells are sometimes preferred for recombinant production because they can appropriately glycosylate and properly fold proteins.

Disclosure of the Invention

In one embodiment, the invention provides a method for treating an individual for a medical condition associated with TGF- β excess. The method provides for the parenteral, oral or local administration of a sufficient amount of TGF- β -binding receptor fragment to the individual to reduce excess TGF- β activity in the individual.

In another embodiment, the method of the present invention provides for the administration of a fragment of human recombinant TGF- β receptor.

In yet another embodiment, the method of the present invention provides for TGF- β receptor fragment administration by intravenous, intraocular, intraarticular, transdermal and enteral methods.

5 In another embodiment, the method of the present invention provides for the administration of the Type II TGF- β receptor. In another embodiment, the administered protein is a fragment of Type II TGF- β receptor. In a further embodiment, the administered
10 protein is Type I TGF- β receptor; in another embodiment, the protein is a Type I TGF- β receptor fragment. In yet another embodiment, Type III TGF- β receptor fragment is administered.

In another embodiment, the TGF- β receptor
15 fragment is administered to patients with cancer. In further embodiments, the type of cancer is plasmacytoma, glioblastoma, or prostatic or ovarian carcinoma.

In another embodiment of the present invention, the TGF- β receptor fragment is administered to patients
20 with collagen vascular diseases, such as systemic sclerosis, polymyositis, scleroderma, dermatomyositis, or systemic lupus erythematosus.

In another embodiment of the present invention, the TGF- β receptor fragment is administered to patients
25 with fibroproliferative disorders. In a further embodiment, the TGF- β receptor fragment is administered to patients with hepatic, intraocular and pulmonary fibrosis. In a further embodiment, the TGF- β receptor fragment is administered to patients with diabetic
30 nephropathy, glomerulonephritis, proliferative vitreoretinopathy, rheumatoid arthritis, liver cirrhosis, and biliary fibrosis.

In still another embodiment, the method of the present invention provides for treating a wound in an
35 individual to avoid excessive connective tissue formation

which is associated with TGF- β excess. The method provides for administration of a sufficient amount of TGF- β -binding receptor fragment to the individual to reduce the excess of TGF- β in the individual. In further
5 embodiments, the types of wounds include surgical incisions, trauma-induced lacerations and wounds involving the peritoneum for which the excessive connective tissue formation is abdominal adhesions. In a
10 further embodiment, the excessive connective tissue formations which are avoided include scars, including those where the scar involves restenosis of blood vessels, and hypertrophic scars, and keloids.

In another embodiment of the present invention, the method provides for administration of TGF- β receptor
15 fragment in the condition of TGF- β excess characterized by immunosuppression associated with an infectious disease. In a further embodiment, the immunosuppression may be associated with trypanosomal infection or viral infections such as human immunosuppression virus, human
20 T cell lymphotropic virus (HTLV-1), lymphocytic choriomeningitis virus and hepatitis.

In another embodiment, the invention provides a method of increasing the effectiveness of a vaccine. In this aspect, TGF- β -binding receptor fragment is
25 administered to an individual about to receive a vaccine or receiving a vaccine. The amount of TGF- β -binding receptor fragment is sufficient to increase the individual's immune response to the vaccine. In a preferred embodiment, the vaccinated individual is
30 immunocompromised.

In another embodiment, the invention provides a method of preventing postradiation fibrosis in an individual undergoing or about to undergo radiation therapy. TGF- β -binding receptor fragment is administered
35

in an amount sufficient to prevent excessive fibrous tissue formation.

Brief Description of the Drawings

5 Figure 1 is a photograph which illustrates binding of biotinylated TGF- β 2 to filter paper embedded with Type II soluble TGF- β receptor fragment (s β -RII).

 Figures 2A and 2B are photographs of electrophoresis results showing that s β -RII from *E. coli* and COS cells, respectively, binds with specificity to TGF- β in solution.

 Figures 3A and 3B show the reaction of antibody specific for a fragment of the s β -RII with *E. coli* soluble and inclusion body fractions (Figure 3a) and with COS supernatants (Figure 3b) transfected with control vector (right lane) or s β -RII vector (left lane).

Modes For Carrying Out the Invention

20 The following terms are used herein:

 "Individual" means a living organism, including humans, other mammals and any other animals which produce TGF- β .

25 "TGF- β " is a family of peptide growth factors, including five members, numbered 1 through 5.

 "TGF- β excess" as used herein is an amount of TGF- β present in serum or tissue which is significantly above the normal level. More preferably, TGF- β excess is a level between about 2 and about 20 times normal. Even more preferably, TGF- β excess is a level between about 2 and about 15 times normal. For example, Deguchi measured 24-hour TGF- β production of bronchoalveolar cells and reported normal levels of 410 ± 225 pg/ 10^7 cells against excess TGF- β production of 1288 ± 453 pg/ 10^7 cells in

systemic lupus erythematosus and 1417 ± 471 pg/ 10^7 cells in scleroderma ((1992) Ann. Rheum. Dis. 51:362-65).

TGF- β excess can be determined, in combination with normal levels, by measurement of the TGF- β protein, of
5 TGF- β mRNA, or of products whose synthesis is stimulated by TGF- β , such as collagen.

TGF- β receptors are cell surface proteins, of which three (Type I, Type II and Type III) are known in mammals.

10 TGF- β receptor Type II is a membrane-bound protein with an intracellular domain, transmembrane domain and extracellular portion which binds to TGF- β . Human TGF- β receptor Type II has been determined to have the amino acid sequence shown in Lin et al., 1992, and
15 corrected by personal communication as shown in SEQ ID NO:1.

A TGF- β receptor fragment is a portion or all of a TGF- β receptor molecule which is capable of binding TGF- β . Preferably, this fragment has a high affinity for
20 TGF- β . Even more preferably, the TGF- β receptor fragment has a greater affinity for TGF- β than does anti-TGF- β antibody or decorin.

"s β -RII" refers to protein fragments of the extracellular portion of the TGF- β receptor Type II which
25 are soluble and bind with high affinity to TGF- β . Preferably, the affinity is in the range of about 10^{-11} to 10^{-12} M, although the affinity may vary considerably with fragments of different sizes, ranging from 10^{-7} to 10^{-13} M. These fragments are proteins consisting of
30 about 136 amino acids or less. Most preferably, s β -RII is about 136 amino acids.

In another embodiment, s β -RII is about 10-110 amino acids in length and comprises the TGF- β binding site. Preferably, the s β -RII of this embodiment is a
35 protein of about 50-80 amino acids.

If the entire native amino acid sequence of 136 amino acids is used, the amino acid sequence resembles that of the entire extracellular portion of the Type II receptor. When smaller $s\beta$ -RII fragments are employed, 5 they resemble various portions of the extracellular portions of the Type II TGF- β receptor, so long as they bind TGF- β with high affinity.

Although the sequence of $s\beta$ -RII is based on the native TGF- β receptor II extracellular fragment, the 10 definition of $s\beta$ -RII also comprises analogs of $s\beta$ -RII which have high affinity for TGF- β . Such analogs include those made by conservative substitutions of amino acids, as well as those made by mutated cells synthesizing $s\beta$ -RII. Only analogs with high affinity for TGF- β are 15 included in this definition.

"Connective tissue" is fibrous tissue characterized by the presence of fibroblasts and fibrous proteins such as collagen and elastin.

A "therapeutic composition" as used herein is 20 defined as comprising $s\beta$ -RII and other physiologically compatible ingredients. The therapeutic composition may contain excipients such as water, minerals and carriers such as protein.

"A sufficient amount of TGF- β -binding receptor 25 fragment" as used herein refers to the amount of TGF- β receptor fragment that neutralizes the biologic activity of excess TGF- β . It may be determined by (1) suitable clinical variables of improvement, (2) pathologic evaluation of the effects on fibrosis and/or 30 immunosuppression or prevention of fibrosis, or (3) a direct inhibition of TGF- β .

This invention provides for administering to an individual with a medical condition associated with TGF- β excess a sufficient amount of TGF- β -binding receptor 35 fragment, such as $s\beta$ -RII, to reduce excess TGF- β activity

in the individual. The TGF- β -binding receptor fragment is all or only a portion of a receptor which is capable of binding TGF- β . s β -RII is made by synthesizing the extracellular domain of the TGF- β Type II receptor

5 (s β -RII) and developing a fragment of this s β -RII domain as a high affinity, soluble binding protein (s β -RII) for TGF- β . This invention further provides for delivering s β -RII to a site where TGF- β is in excess, such as in disease states characterized by fibroproliferation and

10 immunosuppression such as is associated with infectious disease.

The s β -RII of the present invention may be synthesized by means known in the art. The longer, 136-amino acid version is preferably synthesized by

15 recombinant techniques which are known to those in the art. Alternately, and preferably for shorter versions of s β -RII, s β -RII also can be synthesized by solid-phase synthetic methods known to those in the art.

While not wishing to be bound by any particular

20 theory, it appears that the s β -RII regulates TGF- β activity by competing for TGF- β with cell-surface receptors. It is further believed that s β -RII inactivates TGF- β by removing it from the free pool of TGF- β available to interact with cell surface receptors.

25 Depending on the pharmacologic properties of clearance, the s β -RII/TGF- β complex is removed from the site of TGF- β excess. This complexing with excess TGF- β reduces the amount of free TGF- β . With less TGF- β available to complex with cell receptors, TGF- β induced

30 fibroproliferation slows down, resulting in stasis of the disease state.

The s β -RII fragments of the present invention may be used to treat viral infections in which there is an overproduction of TGF- β and immunosuppression.

35 Examples of viruses with which TGF- β excess is associated

include, but are not limited to, hepatitis C, lymphocytic choriomeningitis, human immunodeficiency virus (HIV), and human T cell lymphotropic virus (HTLV-1), the latter being discussed in Kim et al. ((1991) Mol. Cell. Biol. 11:5222-28).

The s β -RII fragment of the present invention may be used to treat the trypanosome-mediated immunosuppression. This may be caused by *Trypanosoma cruzi* or *Leishmania cruzi*, among others.

Immunosuppression in *Leishmania cruzi* has been studied by Barral-Netto et al., (1992) Science 257:545-48.

The s β -RII of the present invention also may be used to increase the efficacy of vaccines. Because TGF- β may cause immunosuppression, the administration of s β -RII can counteract immunosuppression caused by TGF- β and increase the vaccine recipient's immune response to the vaccine. s β -RII should be particularly effective in immunosuppressed patients. s β -RII may be administered before or concomitantly with the vaccine.

The s β -RII fragments of the present invention also may be used to treat forms of cancer which are associated with excess TGF- β . It is also generally known that TGF- β is produced by different tumor cells (sarcomas and carcinomas). Specific examples of tumors in which TGF- β production is excessive include glioblastoma (Wrann et al. (1987) EMBO. J. 6: 1633-36; and Bodner et al. (1989) J. Immunol. 143: 3222-29) and plasmacytoma (Berg and Lynch, (1991) J. Immunol. 146: 2865-72). This TGF- β production may protect the tumor cells from recognition by the host's immune system, Wrann et al. (1987) EMBO. J. 6:1633. In these situations, TGF- β suppresses the proliferation of T and B cells, NK cells, LAK cells, and macrophages that are normally involved in tumor destruction. The s β -RII fragments of the present invention also may be used to treat prostatic cancer.

Merz et al. ((1991) Mol. Endocrin. 5:503-13) reported that elevated TGF- β is correlated with progression of prostatic hypertrophy to malignancy and to metastasis. Steiner and Barrack have also reported that

5 overproduction of TGF- β 1 prostatic tumors produced more extensive metastatic disease ((1992) Mol. Endocrin. 6:15-25)). Therefore, early treatment with s β -RII may help abort progression to malignancy; and later treatment may prevent metastasis.

10 The administration of s β -RII fragments of the present invention may be used in fibroproliferative disorders. As mentioned above, animal models of glomerulonephritis have shown good results with anti-TGF- β antibodies blocking excess TGF- β . These
15 antibodies will be difficult to deliver because they have a high molecular weight and they may result in severe allergic reactions when they are derived from other species. Thus, it would be preferable to administer a lower molecular weight, native protein or close analog,
20 such as s β -RII, in glomerulonephritis. Kidney diseases associated with TGF- β excess include, but are not limited to, mesangial proliferative glomerulonephritis, crescentic glomerulonephritis, diabetic nephropathy, renal interstitial fibrosis, renal fibrosis in transplant
25 patients receiving cyclosporin, and HIV-associated nephropathy. These conditions are associated with excessive fibrous tissue formation which administration of s β -RII should suppress.

30 As s β -RII by itself is not known to have an effect aside from capturing TGF- β , s β -RII may safely be administered during or at the end of retinal reattachment surgery, which is the most common cause of proliferative vitreoretinopathy (PVR) (Connor et al. (1989) J. Clin. Invest. 83:1661-1666).

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Another important fibroproliferative condition is rheumatoid arthritis (RA), which is also associated with excess TGF- β production. Data indicate that blocking TGF- β at any time in the development or chronic stages of RA may help stop the progressive deterioration of the joint and bone. Hence, s β -RII fragments of the present invention may be administered to patients with early joint pain and to patients with prolonged joint pain and deteriorated joints. The current theory is that joint deterioration in RA is due to an overproduction of TGF- β . Excess TGF- β has been measured in joints after test animals are injected with streptococcal cell walls, whose presence is believed to cause RA. Because anti-TGF- β antibody blocks arthritic changes in this model, it is believed that s β -RII may also have a positive effect.

Work in an animal model suggests that chronic liver cirrhosis which is characterized by excess collagen deposition, could be mediated by TGF- β . (Czaja et al. (1989) J. Cell. Biol., 108: 2477-82; and Hoyt et al. (1988) J. Pharm. Exp. Ther. 246: 765). In patients with chronic hepatitis and cirrhosis, the levels of TGF- β 1 mRNA were 2-14 times higher and correlated with higher measurements of serum procollagen than were observed in patients with normal or fatty livers. Six of eight patients with hepatitis C were treated with alpha-interferon for one year and had sustained clinical improvement and normalization of serum procollagen activity. These treated patients also had normal levels of TGF- β 1 mRNA in liver biopsy specimens taken at the end of one year, further supporting the role of TGF- β in liver fibrosis (Castilla et al. (1991) N. Engl. J. Med. 324:933-40).

Cirrhosis of the liver is a widespread condition which is associated with an abnormally high degree of fibrous tissue in the liver and frequently with

high levels of TGF- β . Cirrhosis is the end product of the liver's reaction to many types of injury, including alcohol abuse, exposure to other chemicals, infections (such as hepatitis), intestinal bypass operations and
5 others. In cirrhosis, normal hepatocytes, which produce fibrous blood proteins and clear toxins from the blood, have been replaced by fibrous tissue. In many instances, TGF- β is in excess. In such instances, the s β -RII fragment of the present invention may be used to treat
10 cirrhosis.

s β -RII fragments also may be used to treat biliary cirrhosis, a condition in which the bile ducts become scarred and interfere with the gall bladder emptying its enzymes and digestive juices into the small
15 intestine and hence with the digestion of fats. s β -RII fragments also may be of assistance in treating this condition when it is associated with excess TGF- β .

Other conditions associated with excess TGF- β levels include idiopathic pulmonary fibrosis and
20 myelofibrosis. To complex with excess TGF- β and to slow the development of excess fibrous tissue, s β -RII is intended for administration in these conditions.

The s β -RII fragments of the present invention may be used to treat collagen vascular diseases that are
25 associated with overproduction of TGF- β . It is currently believed that there is an overproduction of TGF- β in collagen vascular diseases, such as progressive systemic sclerosis (PSS), polymyositis, scleroderma, dermatomyositis, eosinophilic fascitis, and morphea.
30 Collagen vascular diseases may also be associated with the occurrence of Raynaud's syndrome. Among other effects, excess TGF- β production may also be involved in interstitial pulmonary fibrosis, an end-stage lung disease which is associated with autoimmune diseases such
35 as systemic lupus erythematosus (SLE) and scleroderma

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(Deguchi, (1992) Ann. Rheum. Dis. 51:362-65); or it may be caused by chemical contact, allergies to dust and hay fever. A therapeutically effective amount of the $s\beta$ -RII of this invention may be administered to neutralize the biologic activity of excess TGF- β , which in turn would prevent unwanted fibrosis.

$s\beta$ -RII fragments of the present invention also may be used in preventing excessive scarring in patients who are known to form keloids or hypertrophic scars. $s\beta$ -RII may be administered to prevent scarring or excessive scarring during healing of various types of wounds including surgical incisions and traumatic lacerations. $s\beta$ -RII may be applied to skin wounds before they are closed to help in healing without scar formation. $s\beta$ -RII also may be placed in surgical abdominal wounds to help prevent adhesion formation which occurs all too commonly after that type of surgery. Williams et al. ((1992) J. Surg. Res. 52:65-70) recently reported that TGF- β was more effective in promoting postoperative peritoneal adhesions than a control of diluent. Intraperitoneal injections of TGF- β for five days did not induce adhesions in unoperated rats. Williams et al. proposed that preventing TGF- β production postoperatively might help prevent adhesion formation. Rather than preventing TGF- β production, which could have systemic side effects, the present invention provides for the local administration of sufficient $s\beta$ -RII to complex with local TGF- β overproduction and prevent excessive healing processes.

According to Lindholm et al., ((1992) J. Cell. Biol. 117:395-400), TGF- β 1 is a strong inhibitor of astrocyte proliferation and may thus interfere with nerve regeneration. Because $s\beta$ -RII complexes with TGF- β , $s\beta$ -RII may encourage nerve regeneration. In deeper wounds

where nerves are cut, the application of $s\beta$ -RII also can help nerve regeneration.

TGF- β excess also has been reported in nasal polyposis, a condition characterized by multiple polyps (Ohno et al. (1992) J. Clin. Invest. 89: 1662-68). $s\beta$ -RII can help lower the TGF- β excess and slow the hyperproliferation that results in polyps. $s\beta$ -RII can be administered after polyp surgery to prevent excessive scarring and recurrence of polyps. $s\beta$ -RII can also be administered to inhibit polyp formation in the intestine.

$s\beta$ -RII may also be administered following coronary angioplasty, preferably placed along the inside of the affected arteries. According to Karas et al., ((1991) Clin. Cardiol. 14:791-801) restenosis or scarring and reclosing of arteries following coronary angioplasty is seen in approximately one-third of patients operated on. Because the fibrous network which ultimately develops into a scar normally accumulates rapidly, early administration of $s\beta$ -RII would reduce excess TGF- β in this area and slow excessive proliferation of connective tissue and restenosis.

TGF- β excess has also been observed in cardiac fibrosis after infarction and in hypertensive vasculopathy. To aid in proper healing without excess scar or fibrous tissue formation, $s\beta$ -RII can be administered in these conditions.

TGF- β excess also has been observed in the tissues of patients receiving radiation therapy. Such tissue is characterized by excess connective tissue development, epithelial thinning and blood vessel occlusion associated with overgrowth of endothelial cells. Administration of $s\beta$ -RII will complex with the excess TGF- β and will contribute to healing without excessive fibrosis.

Formulation, Administration and Dosage

The formulation, method of administration and dosage s β -RII will depend upon the disorder to be treated, and the medical history of the patient. These factors are readily determinable in the course of therapy. Suitable patients with conditions caused by an excess of TGF- β can be identified by laboratory tests, medical history and physical findings. TGF- β excess can be determined directly by immunoassay (Example 6 below) of the patient's serum or of the affected tissue. Excess TGF- β can also be determined by bioassays such as the cell proliferation assay described in Kekow et al., (1990) Proc. Natl. Acad. Sci. U.S.A. 87: 8321-25. Excess TGF- β also can be determined indirectly by measuring the level of TGF- β mRNA (for example, in the polymerase chain reaction of Kekow et al.).

The medical history may reveal facts which support a diagnosis of fibroproliferative disorder, collagen vascular disease, immunosuppression, or of potential for problematic wound healing, as in peritoneal adhesions following surgery, or restenosis of blood vessels after coronary angioplasty. Conditions which are identified as being associated with high levels of TGF- β and/or proliferation of fibrous tissue are considered to cause TGF- β excess.

Patients may have a wide spectrum of physical findings which are indicative of such disorders. Skin biopsies have been used to test TGF- β in patients with systemic sclerosis. Swollen, hot joints are seen in arthritis.

In accordance with the method of the present invention, the formulation comprises s β -RII in an administrable form. The method of the present invention provides for formulating s β -RII in modes which are readily apparent to those skilled in the art.

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Preferably, the s β -RII is dissolved in physiologically compatible carriers.

Physiologically compatible carriers for s β -RII include intravenous solutions, such as normal saline, serum albumin, 5% dextrose, plasma preparations, other protein-containing solutions and TPN solutions. The preferred carrier for parenteral administration of s β -RII is a sterile, isotonic aqueous solution, such as saline or 5% dextrose. Even more preferred is normal saline with human serum albumin. For use in enhancing the immune response to vaccines, s β -RII may be mixed with the vaccine formulation.

Depending on the mode of administration, the s β -RII composition may be in the form of liquid or semi-solid dosage preparations, such as for example, liquids, suspensions or the like. Alternatively, a solution of s β -RII may be placed into an implant, such as an osmotic pump, for the slow release of s β -RII over an extended period of time. Alternatively, s β -RII may be provided in sustained release carrier formulations such as semi-permeable polymer carriers in the form of suppositories or microcapsules. See, for instance, U.S. Patent No. 3,773,919 for Microcapsular Sustained Release Matrices Including Polylactides; Sidmon et al., Biopolymers 22 (1), 547-556 (1983) for copolymers of L-glutamic acid and γ -ethyl-L-glutamate; Langer et al., J. Biomed. Res. 15, 167-277 (1981) for poly(2-hydroxyethylmethacrylate) or the like. Finally, receptor fragmentation and modifications, such as fusion of the s β -RII fragment with human immunoglobulin (IgG) or with polyethylene glycol (PEG) so as to extend the half life of the s β -RII fragment, are other alternative forms of administration.

The mode of administration delivers s β -RII to the individual in a safe, physiologically effective manner. s β -RII may be given by intraocular, intranasal,

subcutaneous, intravenous, intramuscular, intradermal, intraperitoneal, intraarticular, enteral or other conventional routes of administration.

5 In a preferred embodiment, the $s\beta$ -RII of the invention is administered locally to the affected tissue sites by bolus injection or perfusion. For example, for PVR, the preferred mode of administration is a single intraocular injection. Local administration is also preferred in peritoneal wounds to avoid adhesion
10 formation and in other wounds to encourage healing with no keloids or visible scars. For nasal polyposis, nasal drops are preferred.

Local and systemic administration are equally preferred in lung fibrosis (parenteral injection or nasal
15 spray or drops) and cancer. In early, localized tumors, localized administration may be preferred. In later tumor stages, where cancer cells may have metastasized, parenteral administration may be preferred, alone or in combination with local injection. RA can be treated by
20 intraarticular or systemic administration.

Systemic administration is the preferred mode of administration in glomerulonephritis, liver cirrhosis, immunosuppressive conditions (such as viral infections, AIDS and trypanosomal infections), and in widespread skin
25 diseases (such as progressive systemic sclerosis, diffuse fascitis, and generalized morphea). Systemic administration also is preferred when $s\beta$ -RII is used to enhance vaccine response. $s\beta$ -RII can be administered with the vaccine by subcutaneous, intramuscular or
30 intradermal injection.

The dose of $s\beta$ -RII to be administered can be readily determined by those skilled in the art, based on the usual patient symptoms discussed above. The dosage of $s\beta$ -RII to be given in a bolus injection is preferred
35 to be between 20 ng and 300 mg. The bolus injection may

be repeated over several days, or the $s\beta$ -RII can be continuously infused. If given as an intravenous infusion, the amount of $s\beta$ -RII to be infused over a 24-hour period is about 1 mg to about 100 mg.

5 The amount of $s\beta$ -RII to administer may also be determined by maintaining the local tissue concentration of TGF- β at a subnormal level, or about 1-1,000 μ g/ml. For tumors, the amount administered is preferably about 20 ng to 300 mg $s\beta$ -RII per gram tumor tissue.

10 Preferably, $s\beta$ -RII is applied topically, injected at the site of the problem or injected intravenously. Most preferably, $s\beta$ -RII is administered by bolus injection at the site where TGF- β is to be controlled. By intravenous injection, $s\beta$ -RII should be
15 administered at a rate to maintain a circulating serum concentration sufficient to reduce the TGF- β excess.

 Preferably, the patient is started with a relatively low dose of $s\beta$ -RII. The low dose preferably should be continued until the patient's acute phase is
20 ameliorated or adequately improved, as indicated appropriate physical findings and laboratory results. Such improvement may be evident in two to three weeks. In the absence of significant improvement, the dose of $s\beta$ -RII should be increased.

25 For patients to be vaccinated, the dose of $s\beta$ -RII is preferably between 20 ng and 300 mg. Preferably, more $s\beta$ -RII is given to immunocompromised vaccinated patients. $s\beta$ -RII can be administered a short time before the vaccine, to permit $s\beta$ -RII to complex with TGF- β prior
30 to vaccination. Or $s\beta$ -RII can be administered simultaneously with the vaccine.

 The invention has been disclosed by direct description. The following examples show that the $s\beta$ -RII
35 binding protein fragment can treat conditions

characterized by an excess of TGF- β ; however, these examples should not be taken in any way as limiting the scope of the method.

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EXAMPLESExample 1

s β -RII was expressed in *E. coli* as a 15 kd
5 protein of 136 amino acids with no carbohydrate. Those
skilled in the art are familiar with cloning genes in the
fashion detailed in Lin et al. ((1992) Cell 68: 775-785)
Lin et al. also disclose the complete amino acid and
nucleotide sequences.

10 The expression vectors used in this work were
prepared from pET3b supplied by W. Studier. A new
vector, pETX, was prepared and differs from pET3b in that
it contains a modified oligonucleotide linker downstream
of the unique BamHI site in the vector, having the
15 sequence 5'...GGATCCCGTGGAGGATTAAACCATGGATGGATGCATAAGCTT
CGAATTC...3' (SEQ ID NO:2).

In addition, the restriction fragment between
the unique EcoRI site and the EcoRV site downstream of
the terminator was deleted so that both restriction sites
20 were destroyed.

The pDJ12833 vector backbone was derived from
pETX by reconstituting the tetracycline resistance gene
and inserting a 385 bp fragment carrying the par locus of
pSC101 (according to the method of Meacock and Cohen,
25 (1980), Cell 20: 529-42) into the unique PvuII site of
pBR322 backbone present in pET3b, the parent vector.
pER10088 is similar to pDJ12833 but does not carry the
par locus. Both vectors contain, in addition, the
translational coupler described in Squires, et al. (1988)
30 J. Biol. Chem. 263:16297-302.

The DNA encoding the extracellular domain of
the type II receptor was subcloned after PCR
amplification of the 136 codons of that domain from a
pre-existing cDNA clone, pH2-3FF (obtained from MIT).
35 The oligonucleotides used in the amplification were

5'...GGGGATCCGATAGTGGAGGATGATTAAATGATCCCACCGCACGTTTCAGAAGT
...3' (5' oligo) (SEQ ID NO:3); and
5'GGGGAATTCAAGCTTAGTCAGGATTGCTGGTGT TATATTCTTCTGA...3'
(3' oligo) (SEQ ID NO:4). Amplification was for 40
5 cycles with annealing at 55°C. The single major product
of this amplification was purified using the Mermaid Kit
from Bio 101 (La Jolla, CA) and digested with BamHI and
EcoRI, gel purified and ligated to pETX vector digested
with the same enzymes + calf alkaline phosphatase. After
10 transformation of JM109, the structure of a single
recombinant plasmid (pDJ16902) was confirmed by DNA
sequencing.

pDJ16919 was constructed by transferring the
expression cassette from pDJ16902 into the backbone of
15 expression plasmid pDJ12833.

The β -RII protein was obtained by growing
E. coli strain W3110 DE3 containing pDJ16919 at 37°C in a
Biostat E fermentor (manufactured by Braun) and inducing
 β -RII synthesis. The cell paste was collected and
20 stored at -80° C until ready for use.

An aliquot of paste was suspended in 0.1 M Tris
pH 8.0, 5 mM EDTA, 1 mM PMSF. Lysozyme was added to a
final concentration of 0.2 mg/ml and the sample incubated
at 4°C for approximately 30 min. The sample was
25 sonicated with 3-60 sec pulses on ice on a Branson 250
Sonifier and centrifuged at 10,000 rpm on a Sorvall
(Wilmington, DE) centrifuge. The pellet was collected,
and this fraction is known as the "inclusion body
fraction".

30 The inclusion body fraction was solubilized in
6 M guanidine HCl and dialyzed against 10 mM HCl. The
dialyzate was neutralized with 1M NaOH and
chromatographed on a Q-Sepharose column (Pharmacia,
Piscataway, NJ). The bound material was eluted with a
35 salt gradient (0-0.5 M sodium chloride) in 0.1 M Tris pH

7.5 and the fractions were analyzed on 18% SDS-PAGE with Coomassie blue stain. The bulk of the $s\beta$ -RII appeared in fractions 5-15 and was pooled for analysis or further purification.

- 5 The $s\beta$ -RII protein prepared in this example was found to bind TGF- β when $s\beta$ -RII was bound to a hydrophobic support, as discussed in Example 2, and in solution as discussed in Example 3.

10 Example 2

- $s\beta$ -RII had been previously solubilized from inclusion bodies in 6M guanidium hydrochloride with 25mM dithiothreitol. The sample was diluted five fold and split into two fractions. The "control" sample was left
15 untreated. The " $s\beta$ -RII" sample was brought to a final concentration of 5mM cystamine and incubated overnight at 4°C. Both samples were dialyzed against 0.1M Tris, concentrated, and applied to a membrane for a ligand blotting assay as follows. First, Immobilon P membrane
20 (Millipore Corp., Bedford MA) was soaked in methanol for five seconds and then in tris-buffered saline (50 mM Tris, pH 7.5, 0.2 NaCl) (TBS) for one to ten minutes. Meanwhile, the dot-blot apparatus (Gibco-BRL, Gaithersburg, MD) was set up and the membrane inserted on
25 top of a sheet of 3MM paper (Whatman International, Ltd., Maidstone, UK) wetted with TBS. This assembly was tightened.

- Fifty μ l TBS was placed in each well. Next, samples of $s\beta$ RII and control were added in any volume up
30 to 200 μ l and vacuum filtered very slowly for about 5-15 minutes.

 The assembly was disassembled, the filter was marked and blocked in TBS with 5% dried milk overnight at 4° C. It is important to start with a fresh TBS-milk

suspension.. Alternately, the filter could also be blocked for one hour at room temperature.

One blot was incubated with 50 pM biotinylated TGF- β 2 (1.25 ng/ml) alone while the other blot was
5 incubated with 50 pM biotinylated TGF- β 2 and 50 nM TGF- β 2(+) as a competing ligand for 1.5 hours at 37°C. Next the filter was washed three times, 10 minutes each with TBS/0.05% Tween 20. Next streptavidin-HRP (Zymed
Laboratories, Inc., South San Francisco CA) was added at
10 a dilution of 1:1000 in TBS/5% milk/0.05% Tween 20. This was incubated at room temperature for 30 minutes. The filter was washed three times, 10 minutes each with TBS/0.05% Tween 20.

The filter was moved to a new dish. It was
15 overlaid with about 6 ml of a 1:1 mixture of ECL reagents (Amersham Corp., Arlington Heights, IL) and incubated for one minute.

The filter paper was blotted on a paper towel and was placed in a plastic bag and sealed. The filter
20 was exposed to film (XAR-5, Eastman Kodak, Rochester, NY) for one to 45 minutes.

The results of the s β -RII fragment binding to biotinylated TGF- β 2 bound to filter paper are shown in
Figure 1.

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Example 3

This example demonstrates that s β -RII binds with TGF- β in solution. Q-Sepharose bound material (804 μ g/ml) was incubated overnight at 4°C with 50 pM 125 I-
30 TGF- β 1 without (-) or with (+) 40 nM unlabeled TGF- β 1. The complexes were cross-linked at 4°C for 15 min with 0.3 mM disuccinimidyl suberate and electrophoresed under reduced conditions on 18% SDS-PAGE. The gel was dried and complexes were visualized by autoradiography. Figure
35 2A is a photograph of these results. Molecular size

standards are indicated at the left in kilodaltons. The
"*" indicates monomeric TGF- β 1; the "***" indicates
dimeric TGF- β 1; the "→" indicates the appropriate size
complex at approximately 31 kD (monomer of TGF- β cross-
5 linked to s β -RII).

Example 4

Plasmid H2-3FF containing a 4.5 kb cDNA
fragment of the human TGF- β type II receptor (Lin et al.,
10 (1992) Cell, 68:775-85) was cloned into the EcoRI site of
TGF- β plasmid pcDNAI was digested with EcoRI. The 4.5 kb
cDNA fragment was isolated and subcloned into the EcoRI
site of plasmid BlueScript SKII⁺. The plasmid obtained
from this subcloning experiment was named BS/ β RII RI.
15 This plasmid was used to transform *E. coli* strain CJ236
to obtain single-stranded DNA containing uracil residues
(UssDNA). Single-stranded uracil-containing DNA was
isolated by infecting CJ236 cells containing plasmid
BS/ β RII RI with helper phage VCS-M13 and subsequent
20 kanamycin selection. The UssDNA obtained from these
cultures was to be used as a template for site-directed
mutagenesis experiments.

An oligonucleotide was synthesized with the
antisense sequence of the TGF- β type II receptor from
25 nucleotides 553-583 (nucleotides are numbered according
to Lin et al., 1992) with the exception of the codon for
Asn¹⁰⁶ which was changed such that a stop codon would be
inserted. The sequence of the oligonucleotide used to
create this mutation is
30 5'-TAGCAACAAGTCAGGTTAGCTGGTGTATATTC-3' (SEQ ID NO:5).
This primer in combination with the UssDNA described
above was used to carry out an *in vitro* mutagenesis
experiment (Kunkel et al., (1985) Proc. Natl. Acad. Sci.
USA 82:488-492). Clones containing the desired mutation
35 were identified by nucleotide sequencing. A clone

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containing a stop codon in place of Asn¹⁰⁶ was named BS/ β RIIs.

BS/ β RIIs plasmid DNA was purified and digested with EcoRI and BgIII to isolate a 1177 base pair fragment comprising the 5' untranslated sequence and sequences for the extracellular domain (ECD) of the receptor and a portion of the transmembrane domain containing the stop codon created by site-directed mutagenesis. This 1177 base pair EcoRI-BgIII fragment was subcloned into the EcoRI-BgIII site of plasmid pSG5 (Stratagene). This plasmid allows expression of heterologous genes in mammalian cells utilizing a SV₄₀ early promoter and SV₄₀ splice and polyadenylation signals. The plasmid created by this subcloning experiment was called pSG/ β RIIs.

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Example 5

COS-M6 cells were maintained in DMEM high glucose media supplemented with 10% fetal bovine serum and antibiotics. COS-M6 cells were transiently transfected using the DEAE-dextran method as described by Seed et al. (1987) Proc. Natl. Acad. Sci. USA 84:3365-3369. Briefly, plasmids pSG/ β RIIs or pSG5 (negative control) were complexed with DEAE-dextran and added to the cultures for 2 hours. Following this incubation, the cells were glycerol shocked, washed and then allowed to recover for 32 hours. The cultures were then washed three times with serum-free media and allowed to grow for an additional 72 hours in serum-free media. The media were collected, the cell debris was removed by centrifugation and then analyzed for the presence of soluble TGF- β type II receptor expression.

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Example 6

Initially the soluble type II receptor protein (s β -RII) was detected by visualization on a Western blot.

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Supernatants were electrophoresed under reducing conditions on SDS-PAGE and blotted (Towbin et al., (1979) Proc. Natl. Acad. Sci. USA 76:4350-4354). Polyclonal rabbit antisera prepared against a peptide (residues 68-89) were incubated with the blot and the immunoreactive proteins were visualized after developing with an alkaline phosphatase conjugated anti-rabbit IgG and Nitro blue tetrazolium (Figures 3A and 3B). See Example 10 for more detail on the antisera preparation. The peptide antisera detect a single protein from *E. coli* (Fig. 3A) and a heterogeneous set of proteins ranging in size from 24-32 kDa from COS cells (Fig. 3B). Binding of radiolabeled TGF- β to proteins expressed by transfected COS-M6 cells was performed essentially as described previously (Segarini et al. (1989) Mol. Endocrinol. 3:261-272).

Briefly, purified TGF- β was radiolabeled with Na¹²⁵I and incubated with aliquots of the conditioned media from cells transfected with either plasmid pSG/ β RIIs or pSG5 as a negative control. Following affinity labeling, TGF- β /soluble receptor complexes were covalently crosslinked with disuccinimidyl suberate, separated by reducing SDS-PAGE and visualized by autoradiography. Included in some of the binding reactions was a 1000-fold molar excess of unlabeled TGF- β to compete for binding with radiolabeled material. Fig. 2B is the result of such a binding experiment demonstrating the presence of an affinity labeled protein of approximately 40 kda that was not present in vector-only transfected cells. Binding of radiolabeled TGF- β 1 could be effectively blocked by including a 1000-fold molar excess of unlabeled TGF- β 1 (Fig. 2B), lane labeled pSG/ β RIIs⁺) but not TGF- β 2 (data not shown) in the binding reaction mixture.

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Example 7

The effect of $s\beta$ -RII is compared with that of anti-TGF antibody in a glomerulonephritis model. Experimental glomerulonephritis can be induced in rats with a single injection of antithymocyte serum because the glomerular mesangial cells express a thy-1.1 epitope on their surfaces. The experimental lesion is acute mesangial proliferative glomerulonephritis and is characterized by expansion of the mesangial matrix and hypercellularity. The injured cells also express more TGF- β 1 mRNA and TGF- β 1, which in turn stimulates the synthesis of two proteoglycans, biglycan and decorin.

The antiserum is prepared by immunizing a rabbit with a cyclized, synthetic peptide containing residues 78-109 of human TGF- β 1. The anti-TGF- β 1 antiserum is capable of inhibiting binding of TGF- β to cells. (Flanders et al. (1988) Biochemistry 27:739-46)

First, glomerulonephritis is induced in rats by an intravenous injection of antithymocyte serum. Next, for six days, three groups of rats are treated with daily intravenous injections of saline (the negative control group), anti-TGF- β 1 antiserum (the positive control group) or $s\beta$ -RII.

On the seventh day, the animals are sacrificed and slides are made of the kidneys, which are stained with periodic acid-Schiff solution to emphasize the pathological changes. The negative control kidneys have full-blown glomerulonephritis with reddish-pink amorphous fibrous material filling most of the glomerulus. The positive control kidneys have a staining pattern which is similar to a normal glomerulus. The kidney which is treated with $s\beta$ -RII also has a normal appearance, indicating that the $s\beta$ -RII blocks the response due to the secretion of excessive TGF- β .

The extent of glomerular injury can be quantitated by performing glomerular cell counts from 30 randomly selected glomeruli from normal animals and nephritic animals in each group. On day 4, there are fewer cells in glomeruli from antithymocyte-treated rats, presumably because the treatment causes cell lysis. By day 7, there are more cells than normal. The changes in cell counts in the anti-TGF- β 1 and s β -RII group are expected to be the same.

Another measure of the effect of anti-TGF- β 1 and s β -RII on the disease process is to quantitate the amount of extracellular matrix accumulation in the glomeruli. The degree of glomerular matrix expansion is determined as the percentage of each glomerulus occupied by the mesangial matrix according to the method of Raij et al. (1984) Kidney Int. 26: 137-43. The anti-TGF- β 1 and s β -RII kidneys are expected to have similar percentages of mesangial matrix to that in normal kidney, and significantly less mesangial matrix than in the negative control kidneys.

After glomerular injury and simultaneous treatment with anti-TGF- β , the mesangial cells expressed more TGF- β 1 mRNA; however, proteoglycan synthesis is nearly normal with anti-TGF- β 1 and s β -RII.

Example 8

The following compares the action of TGF- β antibody with s β -RII in an arthritis model. TGF- β antibody is prepared as disclosed in U.S. Application Serial No. 759,109, which is incorporated by reference in its entirety. This application discloses the formation of monoclonal antibodies 3C7.14 specific for TGF- β 2 and TGF- β 3 and 1D11.16 specific for TGF- β 1, - β 2 and - β 3.

First, arthritis is induced in pathogen-free female LEW rats (Harlan Sprague Dawley, Indianapolis, IN)

weighing about 100 grams. Each receives a dose of cell wall fragments from Group A streptococci (SCW) (30 μ g rhamnose/gm body weight), injected intraperitoneally (ip) according to the technique described in Brandes et al.

5 (1991) J. Clin. Invest. 87:1108.

SCW-injected and control LEW rats are given an intraarticular (IA) injection in one of the hind ankles of one of the following:

- 10 1. anti-TGF- β (1D11.16) which is specific for TGF- β 1 and TGF- β 1 in 25 μ l PBS,
2. s β -RII in 25 μ l PBS,
3. PBS only, or
4. an irrelevant isotype control mouse myeloma immunoglobulin (MOPC21, IgG₁)

15 Joints are clinically monitored by determining the amount of joint erythema, swelling and distortion on a scale of 0 (normal) to 4 (severe inflammation). Radiographs are taken and are evaluated for soft tissue swelling, joint space narrowing, bone erosions and
20 deformity. Tissue specimens are obtained and prepared for histopathologic analysis as described in Brandes et al., *ibid.* Total RNA is isolated from excised synovial tissues according to the method of Allen et al. (1990) J. Exp. Med. 171:231.

25 Injection of SCW produces an acute inflammatory response which is clinically detectable within hours and maximal in 3-5 days. When anti-TGF- β is injected directly into a joint before ip administration of the SCW, inflammation at 24 hours is significantly below that
30 observed in joints with the irrelevant antibody. At the peak of the acute response, inflammation of anti-TGF- β joints remains far below that of joints with the irrelevant antibody. Even if joints are injected with anti-TGF- β when inflammation is well developed (day 13),
35 anti-TGF- β still has a significant anti-inflammatory

effect, when compared to irrelevant antibody. Because $s\beta$ -RII also binds TGF- β , $s\beta$ -RII has a similarly beneficial effect when given early or late in the inflammatory process.

5

Example 9

To simulate acute liver injury, the hepatotoxin, D-galactosamine, is administered to cause liver fibrosis, mortality, and maximal TGF- β gene
10 expression approximately 48 hours after administration. A rat model utilizing this hepatotoxin is used to evaluate the therapeutic effect of $s\beta$ -RII on acute liver fibrosis and serves as a model for liver cirrhosis.

Sprague-Dawley rats are administered 1.6 g/kg
15 D-galactosamine intraperitoneally. Half of the rats are also to be given $s\beta$ -RII two hours prior to D-galactosamine administration, and at 24, 48, and 72 hours after D-galactosamine administration. Two rats from each test group are sacrificed at 48 hours to
20 evaluate the efficacy of $s\beta$ -RII at peak TGF- β gene expression.

Histological examination reveals that $s\beta$ -RII-treated animals exhibit reduced liver pathology. Northern blot evaluation of tissues from specimens
25 treated with $s\beta$ -RII show significantly decreased levels of collagen mRNA and almost normal levels of serum albumin, in contrast to non-treated controls.

Example 10

30 Polyclonal antibodies to the carboxy-terminal region of $s\beta$ -RII have been prepared and tested. Linear or cyclic peptides of amino acids 68-89 were injected once per month (in one case a month was skipped) at a concentration of 4 mg/ml in phosphate buffered saline
35 with 200 μ l per injection in complete Freund's adjuvant

(first injection) or incomplete Freund's adjuvant (succeeding boosts). Bleeds were collected during the second, fourth, fifth, sixth, and seventh months after the initial boost.

5 Soluble and inclusion body fractions from *E. coli*, and supernatant fractions from s β -RII and vector-transfected COS cells were electrophoresed under reducing conditions on SDS-PAGE. The *E. coli* fractions were prepared in duplicate. The proteins were
10 electrotransferred to Immobilon P filters (Millipore, Bedford MA). Next, the *E. coli* filters were blocked with super-Blotto (2.5% nonfat dry milk in Tris buffered saline (TBS), 10% (v,v) glycerol, 1M glucose, 0.5% Tween 20). The COS filters were blocked with standard Blotto
15 (5% nonfat dry milk in Tris buffered saline). Then all filters were reacted with 10 μ g/ml of rabbit antiserum. One half of the duplicate *E. coli* filters was incubated with nonimmune rabbit antiserum as a control. The blots were washed with TBS, containing 0.05% Tween 20 (T-TBS),
20 and reacted with a secondary antibody, goat anti-rabbit IgG horseradish peroxidase (HRP) diluted 1:50,000, and incubated for one hour. The blot was washed with T-TBS and reacted with a membrane TMB kit for Kirkegaard and Perry according to package directions.

25 Figure 3A shows *E. coli* soluble and inclusion body fractions reacted with peptide antiserum (left lane) or with control rabbit antisera (right lane). Figure 3B shows COS supernatants from cells transfected with control vector (right lane) or s β -RII vector (left lane).
30 s β -RII produced in COS cells is glycosylated and appears as heterogeneous bands.

In both *E. coli* and COS systems, a soluble receptor was produced and included an amino acid sequence that is recognized by antisera induced by linear and/or
35 cyclic peptides of amino acids 68-89 of the extracellular

domain of the type II TGF- β receptor. Control nonimmune rabbit antiserum did not recognize the type II receptor. Vector-transfected COS cells did not produce a protein that is reactive with the antisera.

5 COS cells were transfected with the pSG/ β RII vector or with a control vector (pSG5). After 72 hours, the two supernatants were collected, incubated with 50 pM 125 I-TGF- β 1 without (-) or with (+) 40 nM unlabeled TGF- β 1. The samples were crosslinked with 0.3mM
10 disuccinimidyl suberate (DSS) (Pierce Chemical Co., Rockford IL) and electrophoresed on SDS-PAGE under reducing conditions. The gels were dried and exposed for autoradiography, with the result shown in Figure 2B. A single asterisk (*) indicates monomeric TGF- β 1, a
15 double asterisk indicates dimeric TGF- β 1 and a single arrowhead indicates the TGF- β 1/s β -RII complex. The size and heterogeneous appearance of the complex suggests that the s β -RII from COS cells is glycosylated with 7-10 kD of carbohydrate.

20

Example 11

The efficacy of TGF- β in preventing post-radiation fibrosis can be assessed in patients who require radiation therapy for a tumor, such as
25 adenocarcinoma, prior to bowel resection. At surgery, biopsies of various tissues can be obtained. There are two negative control groups: patients who do not receive radiotherapy prior to surgery and patients who receive radiotherapy but no TGF- β . The study group is
30 administered s β -RII concomitantly with the radiotherapy.

Each day, when the patient reports for radiotherapy, the patient receives an intravenous injection of s β -RII. After radiation therapy is stopped, the patient receives weekly intravenous doses of s β -RII
35 until surgery.

At surgery, the tumor and associated tissues are removed. Slides are made from the tumor and tissue samples. Under microscopic examination, the tissue samples show signs of healing without excessive fibrosis.

5

This invention has been detailed both by example and by direct description. It should be apparent that one having ordinary skill in this art would be able to surmise equivalents to the invention as described in the claims which follow but which would be within the spirit of the description above. Those equivalents are to be included within the scope of this invention.

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- 10 (ii) TITLE OF INVENTION: USES OF TGF-BETA RECEPTOR FRAGMENT AS A
THERAPEUTIC AGENT
- (iii) NUMBER OF SEQUENCES: 5
- (iv) CORRESPONDENCE ADDRESS:
15 (A) ADDRESSEE: Morrison & Foerster
(B) STREET: 755 Page Mill Road
(C) CITY: Palo Alto
(D) STATE: California
(E) COUNTRY: USA
(F) ZIP: 94304-1018
- 20 (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- 25 (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US UNASSIGNED
(B) FILING DATE:
(C) CLASSIFICATION:
- 30 (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: LUTHER, BARBARA J.
(B) REGISTRATION NUMBER: 33,954
(C) REFERENCE/DOCKET NUMBER: 22095-20261.20
- (ix) TELECOMMUNICATION INFORMATION:
35 (A) TELEPHONE: (415) 813-5600
(B) TELEFAX: (415) 494-0792

(C) TELEX: 706141

(2) INFORMATION FOR SEQ ID NO:1:

- 5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2095 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGCTCGTTGG CGAGGAGTTT CCTGTTTCCC CCGCAGCGCT GAGTTGAAGT TGAGTGAGTC
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120
CTCCTGTGCA GCTTCCCTCG GCCGCCGGGG GCCTCCCCGC GCCTCGCCGG CCTCCAGGCC
180
20 CCTCCTGGCT GGCAGCGGG CGCCACATCT GGCCCGCACA TCTGCGCTGC CGGCCCGGCC
240
CGGGGTCCGG AGAGGGCGCG GCGCGGAGCG CAGCCAGGGG TCCGGAAGG CGCCGTCCGT
300
25 GCGCTGGGGG CTCGGTCTAT GACGAGCAGC GGGGTCTGCC ATGGGTCGGG GGCTGCTCAG
360
GGGCCTGTGG CCGCTGCACA TCGTCCTGTG GACGCGTATC GCCAGCACGA TCCACCGCA
420
30 CGTTCAGAAG TCGGTTAATA ACGACATGAT AGTCACTGAC AACAACGGTG CAGTCAAGTT
480
TCCACAACCTG TGTAATTTT GTGATGTGAG ATTTTCCACC TGTGACAACC AGAATCCTG
540
35

- 40 -

CATGAGCAAC TGCAGCATCA CCTCCATCTG TGAGAAGCCA CAGGAAGTCT GTGTGGCTGT
600

ATGGAGAAAG AATGACGAGA ACATAACACT AGAGACAGTT TGCCATGACC CCAAGTCCC
660

5 CTACCATGAC TTTATTCTGG AAGATGCTGC TTCTCCAAAG TGCATTATGA AGGAAAAAAA
720

AAAGCCTGGT GAGACTTTCT TCATGTGTTT CTGTAGCTCT GATGAGTGCA ATGACAACAT
780

10 CATCTTCTCA GAAGAATATA ACACCAGCAA TCCTGACTTG TTGCTAGTCA TATTTCAAGT
840

GACAGGCATC AGCCTCCTGC CACCACTGGG AGTTGCCATA TCTGTCATCA TCATCTTCTA
900

15 CTGCTACCGC GTTAACCGGC AGCAGAAGCT GAGTTCAACC TGGGAAACCG GCAAGACCGC
960

GAAGCTCATG GAGTTCAGCG AGCACTGTGC CATCATCTTG GAAGATGACC GCTCTGACAT
1020

20 CAGCTCCACG TGTGCCAACA ACATCAACCA CAACACAGAG CTGCTGCCCA TTGAGCTGGA
1080

CACCCTGGTG GGGAAAGGTC GCTTTGCTGA GGTCTATAAG GCCAAGCTGA AGCAGAACAC
1140

25 TTCAGAGCAG TTTGAGACAG TGGCAGTCAA GATCTTTCCC TATGAGGAGT ATGCCTCTTG
1200

GAAGACAGAG AAGGACATCT TCTCAGACAT CAATCTGAAG CATGAGAACA TACTCCAGTT
1260

30 CCTGACGGCT GAGGAGCGGA AGACGGAGTT GGGGAAACAA TACTGGCTGA TCACCGCCTT
1320

CCACGCCAAG GGCAACCTAC AGGAGTACCT GACGCGGCAT GTCATCAGCT GGGAGGACCT
1380

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-41-

GCGCAAGCTG GGCAGCTCCC TCGCCCGGGG GATTGCTCAC CTCCACAGTG ATCACACTCC
1440

ATGTGGGAGG CCCAAGATGC CCATCGTGCA CAGGGACCTC AAGAGCTCCA ATATCCTCGT
1500

5

GAAGAACGAC CTAACCTGCT GCCTGTGTGA CTTTGGGCTT TCCCTGCGTC TGGACCCTAC
1560

TCTGTCTGTG GATGACCTGG CTAACAGTGG GCAGGTGGGA ACTGCAAGAT ACATGGCTCC
1620

10

AGAAGTCCTA GAATCCAGGA TGAATTTGGA GAATGCTGAG TCCTTCAAGC AGACCGATGT
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CTACTCCATG GCTCTGGTGC TCTGGGAAAT GACATCTCGC TGTAATGCAG TGGGAGAAGT
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AAAAGATTAT GAGCCTCCAT TTGGTTCCAA GGTGCGGGAG CACCCCTGTG TCGAAAGCAT
1800

GAAGGACAAC GTGTTGAGAG ATCGAGGGCG ACCAGAAATT CCCAGCTTCT GGCTCAACCA
1860

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CCAGGGCATC CAGATGGTGT GTGAGACGTT GACTGAGTGC TGGGACCACG ACCCAGAGGC
1920

CCGTCTCACA GCCCAGTGTG TGGCAGAACG CTTCAGTGAG CTGGAGCATC TGGACAGGCT
1980

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CTCGGGGAGG AGCTGCTCGG AGGAGAAGAT TCCTGAAGAC GGCTCCCTAA AACTACCAA
2040

ATAGCTCTTA TGGGGCAGGC TGGGCATGTC CAAAGAGGCT GCCCCTCTCA CCAAA
2095

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 49 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

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-42-

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5

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49

(2) INFORMATION FOR SEQ ID NO:3:

10

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 52 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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52

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(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 46 base pairs

(B) TYPE: nucleic acid

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(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

30

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46

(2) INFORMATION FOR SEQ ID NO:5:

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(i) SEQUENCE CHARACTERISTICS:

-43-

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TAGCAACAAG TCAGGTTAGC TGGTGTTATA TTC

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CLAIMS

1. A method for treating an individual for a medical condition associated with TGF- β excess comprising
5 administering parenterally, orally or locally a sufficient amount of TGF- β -binding receptor fragment to the individual to reduce excess TGF- β activity in the individual.
- 10 2. The method of claim 1 wherein the TGF- β receptor fragment comprises a fragment of recombinant human TGF- β receptor.
- 15 3. The method of claim 1 wherein the TGF- β receptor fragment is administered by a method selected from the group consisting of intravenous, intraocular, intraarticular, transdermal, and enteral administration.
- 20 4. The method of claim 1 wherein the TGF- β receptor is type II.
5. The method of claim 1 wherein the TGF- β receptor is type I.
- 25 6. The method of claim 1 wherein the TGF- β receptor is type III.
7. The method of claim 1 wherein said medical condition comprises cancer.
- 30 8. The method of claim 7 wherein said cancer is plasmacytoma, glioblastoma, astrocytoma, or prostatic or ovarian carcinoma.

9. The method of claim 1 wherein said medical condition comprises a collagen vascular disease.

10. The method of claim 9 wherein said
5 collagen vascular disease is systemic sclerosis, polymyositis, scleroderma, or dermatomyositis.

11. The method of claim 1 wherein said medical condition comprises an autoimmune disease.
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12. The method of claim 11 wherein said autoimmune disease is rheumatoid arthritis or systemic lupus erythematosus.

13. The method of claim 1 wherein said medical condition comprises a fibroproliferative disorder.
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14. The method of claim 13 wherein said fibroproliferative disorders comprise hepatic, kidney, intraocular and pulmonary fibrosis.
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15. The method of claim 13 wherein said fibroproliferative disorder is selected from the group consisting of diabetic nephropathy, glomerulonephritis, proliferative vitreoretinopathy, liver cirrhosis, biliary fibrosis, and myelofibrosis.
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16. The method of claim 1 wherein the condition of TGF- β excess is characterized by immunosuppression associated with an infectious disease.
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17. The method of claim 16 wherein the immunosuppression occurs with a trypanosomal infection.

18. The method of claim 16 wherein the immunosuppression occurs with a viral infection selected from the group consisting of human immunosuppression virus, HTLV-1, lymphocytic choriomeningitis virus, and hepatitis.

19. A method of increasing the effectiveness of a vaccine comprising administering to an individual about to receive a vaccine or receiving a vaccine a sufficient amount of TGF- β -binding receptor fragment to increase the immune response to the vaccine in the individual.

20. The method of claim 19 wherein the individual is an immunocompromised individual.

21. A method of treating a wound in an individual to avoid excessive connective tissue formation associated with TGF- β excess, the method comprising administering a sufficient amount of TGF- β -binding receptor fragment to the individual to reduce the excess of TGF- β in the individual.

22. The method of claim 21 wherein the wound is a surgical incision or trauma-induced laceration.

23. The method of claim 21 wherein the wound involves the peritoneum and the excessive connective tissue formation comprises abdominal adhesions.

24. The method of claim 21 wherein the excessive connective tissue is selected from the group consisting of a scar, hypertrophic scar, and a keloid.

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25. The method of claim 24 wherein the scar involves restenosis of blood vessels.

26. A method of preventing postradiation
5 fibrosis in an individual undergoing or about to undergo radiation therapy, the method comprising administering to the individual TGF- β -binding receptor fragment in amounts sufficient to prevent excessive fibrous tissue formation.

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AMENDED CLAIMS

-[received by the International Bureau
on 08 April 1994 (08.04.94);
original claims 1, 19, 21 and 26 amended;
remaining claims unchanged (3 pages)]

1. A method for treating an individual for a medical condition associated with TGF- β excess comprising
5 administering parenterally, orally or locally a sufficient amount of a high affinity TGF- β -binding receptor fragment to the individual to reduce excess TGF- β activity in the individual.
- 10 2. The method of claim 1 wherein the TGF- β receptor fragment comprises a fragment of recombinant human TGF- β receptor.
- 15 3. The method of claim 1 wherein the TGF- β receptor fragment is administered by a method selected from the group consisting of intravenous, intraocular, intraarticular, transdermal, and enteral administration.
- 20 4. The method of claim 1 wherein the TGF- β receptor is type II.
5. The method of claim 1 wherein the TGF- β receptor is type I.
- 25 6. The method of claim 1 wherein the TGF- β receptor is type III.
7. The method of claim 1 wherein said medical condition comprises cancer.
- 30 8. The method of claim 7 wherein said cancer is plasmacytoma, glioblastoma, astrocytoma, or prostatic or ovarian carcinoma.

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18. The method of claim 16 wherein the immunosuppression occurs with a viral infection selected from the group consisting of human immunosuppression virus, HTLV-1, lymphocytic choriomeningitis virus, and hepatitis.

19. A method of increasing the effectiveness of a vaccine comprising administering to an individual about to receive a vaccine or receiving a vaccine a sufficient amount of a high affinity TGF- β -binding receptor fragment to increase the immune response to the vaccine in the individual.

20. The method of claim 19 wherein the individual is an immunocompromised individual.

21. A method of treating a wound in an individual to avoid excessive connective tissue formation associated with TGF- β excess, the method comprising administering a sufficient amount of a high affinity TGF- β -binding receptor fragment to the individual to reduce the excess of TGF- β in the individual.

22. The method of claim 21 wherein the wound is a surgical incision or trauma-induced laceration.

23. The method of claim 21 wherein the wound involves the peritoneum and the excessive connective tissue formation comprises abdominal adhesions.

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24. The method of claim 21 wherein the excessive connective tissue is selected from the group consisting of a scar, hypertrophic scar, and a keloid.

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- 50 -

25. The method of claim 24 wherein the scar involves restenosis of blood vessels.

26. A method of preventing postradiation
5 fibrosis in an individual undergoing or about to undergo
radiation therapy, the method comprising administering to
the individual a high affinity TGF- β -binding receptor
fragment in amounts sufficient to prevent excessive
fibrous tissue formation.

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STATEMENT UNDER ARTICLE 19

Applicant respectfully submits that the above amendment to claim 1 more clearly defines the invention. Support for the added phrase is found on page 10, lines 23-31.

The claimed inventions involve an inventive step because no one reference cited in the International Search Report nor any combination of these references teach or suggest applicant's inventive method. More particularly, applicant's invention is directed to a method for treating an individual with a medical condition associated with excess TGF- β . Applicant's method comprises the administration of a high affinity TGF- β binding receptor fragment in order to reduce the excess TGF- β found in the individual having the medical condition. The cited prior art does not adequately teach or suggest this therapeutic use of a high affinity TGF- β receptor fragment.

More specifically, the Border et al. (1992) reference at best can only be viewed as an invitation to experiment with soluble forms of TGF- β receptors. As stated on page 6, first column, first paragraph of the Border reference, "soluble forms of the receptors may also inhibit TGF- β activity by the same mechanism but this has not yet been proven. (emphasis added) The Smith et al. (1987) reference teaches only the use of soluble CD₄ for binding to the HIV-1 envelope glycoprotein gp120. These results can not be extrapolated to the therapeutic use of TGF- β -receptor fragments. The Andres et al. (1989) and Lin et al. (1992) references do not teach the therapeutic use of TGF- β receptor fragments. While the remainder of the cited references discuss the role of TGF- β in various medical conditions, applicant can not find a teaching or suggestion directed to the therapeutic use of a high affinity TGF- β -binding receptor fragment in these references.

In view of the above, applicant respectfully submits that the claimed inventions contain an inventive step over the prior art.

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FIG. 1

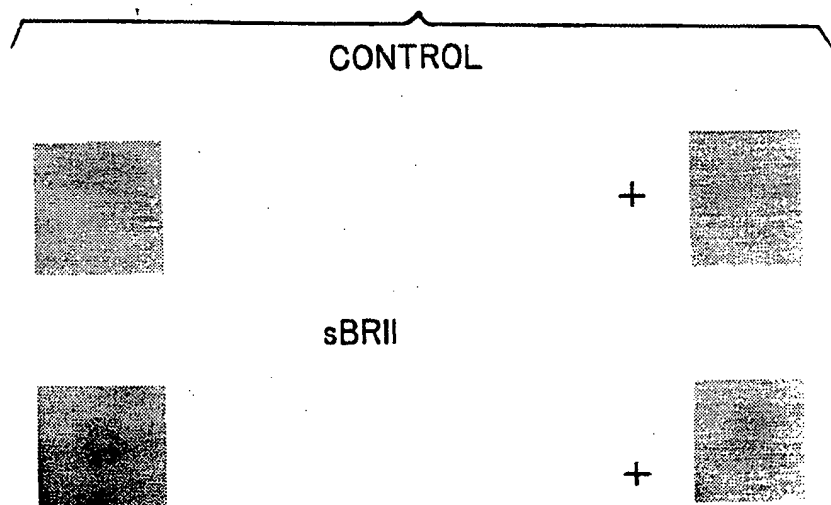
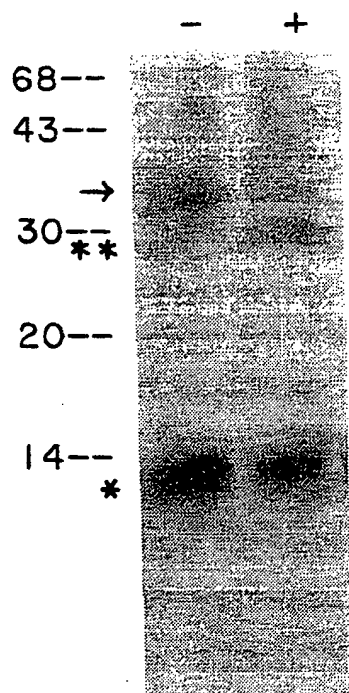


FIG. 2A



2 / 2

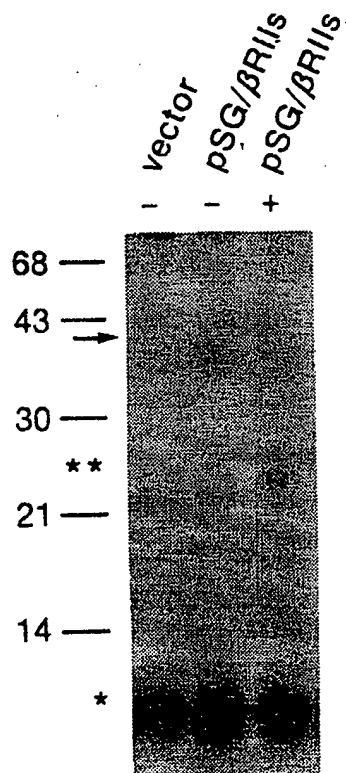


FIG. 2B

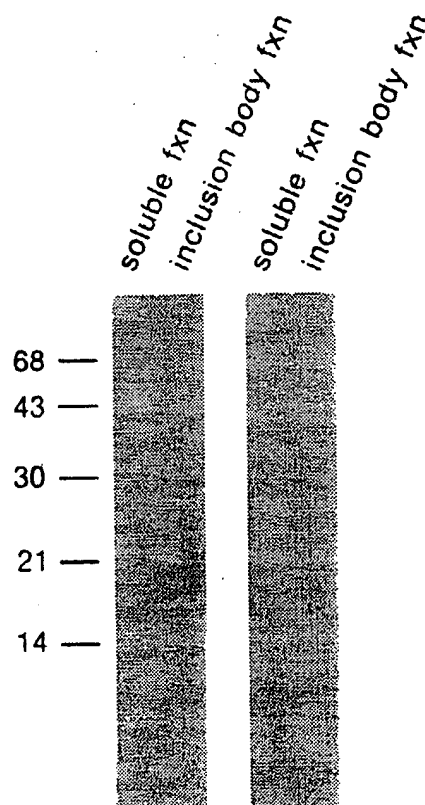


FIG. 3A

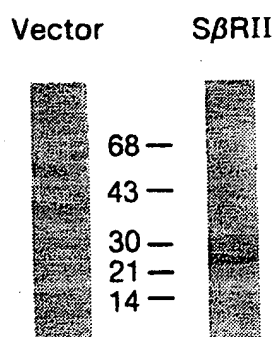


FIG. 3B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10455

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/43, 37/02

US CL :514/2, 8

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched, (classification system followed by classification symbols)

U.S. : 514/2, 8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Journal of Cell Biology, Volume 109, Number 6, Part 1, issued December 1989, J. L. Andres et al., "Membrane-anchored and Soluble Forms of Betaglycan, a Polymorphic Proteoglycan that Binds Transforming Growth Factor-beta", pages 3137-45, especially the abstract.	1-24
Y	Journal of Clinical Investigation, Volume 90, issued July 1992, W. A. Border et al., "Transforming Growth Factor-beta in Disease: The Dark Side of Tissue Repair", pages 1-7, especially page 1, col. 2, and page 5, col.2 to page 6, col. 1.	1-24



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	Z	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

26 January 1994

Date of mailing of the international search report

08 FEB 1994

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

DAVID L. FITZGERALD

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10455

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Science, Volume 238, issued 18 December 1987, D. H. Smith et al., "Blocking of HIV-1 Infectivity by a Soluble, Secreted Form of the CD4 Antigen", pages 1704-07, especially the abstract; page 1704, col. 2; and page 1705, col. 3 to page 1706, col. 1.	1-24
Y	Cell, Volume 68, issued 21 February 1992, H. Y. Lin et al., "Expression Cloning of the TGF-beta Type II Receptor, a Functional Transmembrane Serine/Threonine Kinase", pages 775-85, especially the abstract and Fig. 2.	1-4, 7-24
Y	Cell, Volume 67, issued 15 November 1991, F. Lopez-Casillas et al., "Structure and Expression of the Membrane Proteoglycan Betaglycan, a Component of the TGF-beta Receptor System", pages 785-95, especially pages 791-92.	1-3, 6-24
Y	Cancer Investigation, Volume 9, Number 3, issued 1991, M. A. Liu et al., "Transforming Growth Factor-beta-Mullerian Inhibiting Substance Family of Growth Regulators", pages 325-36, especially Table I.	1-24
Y	Lancet, Volume 336, issued 15 December 1990, G. F. Whalen, "Solid tumours and wounds: transformed cells misunderstood as injured tissue?", pages 1489-92, see the entire document.	1-8
Y	Immunology Today, Volume 10, Number 8, issued 1989, S. M. Wahl et al., "Inflammatory and immunomodulatory roles of TGF-beta", pages 258-61, see the entire document.	1-6, 11, 12, 16-20
Y	Annals of the New York Academy of Sciences, Volume 593, issued 1990, R. L. Wilder et al., "Transforming growth factor-beta in rheumatoid arthritis", pages 197-207, especially the abstract.	1-6, 11, 12
Y	Journal of Cell Biology, Volume 108, issued June 1989, M. J. Czaja et al., "In Vitro and In Vivo Association of Transforming Growth Factor-beta-1 with Hepatic Fibrosis", pages 2477-82, especially the abstract.	1-6, 13, 14, 16
Y	Nature, Volume 346, issued 26 July 1990, W. A. Border et al., "Suppression of experimental glomerulonephritis by antiserum against transforming growth factor beta-1", pages 371-74, especially the abstract.	1-6, 13-15

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10455

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	American Journal of Respiratory Cell and Molecular Biology, Volume 5, issued 1991, N. Khalil et al., "Increased Production and Immunohistochemical Localization of Transforming Growth Factor-beta in Idiopathic Pulmonary Fibrosis", pages 155-62, especially the abstract.	1-6, 13, 14

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/10455

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

DATABASES: USPTO-APS, Medline, Biosis, SciSearch, Derwent WPI

SEARCH TERMS: TGF- or Transforming Growth Factor-Beta; cancer or neoplasm; collagen; autoimmune;
immunosuppress? or immunodeficiency; vaccine or adjuvant; fibrosis or
glomerulonephritis; wound heal? or scar?

